CRITERION™ DNASE TEST AGAR WITH TOLUIDINE BLUE

Cat. no. C5670	CRITERION™ DNase Test Agar with Toluidine Blue	85gm
Cat. no. C5671	CRITErION™ DNase Test Agar with Toluidine Blue	500gm
Cat. no. C5672	CRITERION™ DNase Test Agar with Toluidine Blue	2kg
Cat. no. C5673	CRITERION™ DNase Test Agar with Toluidine Blue	10kg
Cat. no. C5674	CRITERION™ DNase Test Agar with Toluidine Blue	50kg

INTENDED USE

Hardy Diagnostics CRITERION™ DNase Test Agar with Toluidine Blue is recommended for the detection of DNase in gram-negative bacteria, especially *Serratia* spp.

This dehydrated culture medium is a raw material intended to be used in the making of prepared media products, which will require further processing, additional ingredients, or supplements.

SUMMARY

DNase with Toluidine Blue O, developed by Schreier in 1969, is a modification of the formula developed by Jeffries, Holtman, and Guse[7,8] The basal medium is composed of casein and soy peptones which supply necessary nutrients. Sodium chloride is added to maintain osmotic equilibrium. DNA is incorporated into the medium to detect organisms which possess the enzyme deoxyribonuclease, while toluidine blue O serves as the color indicator. The addition of toluidine blue eliminates the need to add HCl to the plate.

The complexing of toluidine blue O with DNA produces a blue color in the uninoculated medium. Organisms which depolymerize DNA result in the formation of a dye, oligonucleotide, and mononucleotide complex. Metachromatic properties of the indicator thereby produce a visible bright rose-pink color in the surrounding area of the organisms possessing the DNase enzyme.

Because toluidine blue may be inhibitory to some gram-positive organisms, it is recommended for the detection of DNase in gram-negative microorganisms.

FORMULA

Gram weight per liter:	42.0gm/L
Pancreatic Digest of Casein	15.0gm
Papaic Digest of Soybean Meal	5.0gm

Sodium Chloride	5.0gm
Deoxyribonucleic Acid	2.0gm
Toluidine Blue	0.1gm
Agar	15.0gm

Final pH 7.3 +/- 0.2 at 25ºC.

* Adjusted and/or supplemented as required to meet performance criteria.

STORAGE AND SHELF LIFE

Store the sealed bottle(s) containing dehydrated culture medium at 2-30ºC. Dehydrated culture medium is very hygroscopic. Keep lid tightly sealed. Protect dehydrated culture media from moisture and light. The dehydrated culture media should be discarded if it is not free-flowing or if the color has changed from its original light bluish-beige.

Store the prepared culture media at 2-8ºC.

The expiration dating on the product label applies to the product in its intact packaging when stored as directed. The product may be used and tested up to the expiration date on the product label and incubated for the recommended quality control incubation times.

Refer to the document "Storage" for more information.

PRECAUTIONS

This product may contain components of animal origin. Certified knowledge of the origin and/or sanitary state of the animals does not guarantee the absence of transmissible pathogenic agents. Therefore, it is recommended that these products be treated as potentially infectious, and handle observing the usual universal blood precautions. Do not ingest, inhale, or allow to come into contact with skin.

This product is for laboratory use only. It is to be used only by adequately trained and qualified laboratory personnel. Observe approved biohazard precautions and aseptic techniques. All laboratory specimens should be considered infectious and handled according to "standard precautions." The "Guidelines for Isolation Precautions" is available from the Centers for Disease Control and Prevention at www.cdc.gov/ncidod/dhqp/gl_isolation.html.

For additional information regarding specific precautions for the prevention of the transmission of all infectious agents from laboratory instruments and materials, and for recommendations for the management of exposure to infectious disease, refer to CLSI document M-29: *Protection of Laboratory Workers from Occupationally Acquired Infections: Approved Guideline.*

Sterilize all biohazard waste before disposal.

Refer to the document "Precautions When Using Media" for more information.

Refer to the document SDS Search instructions on the Hardy Diagnostics' website for more information.

METHOD OF PREPARATION FOR DEHYDRATED CULTURE MEDIA

1. Suspend 42.0gm of the dehydrated culture media in 1 liter of distilled or deionized water. Stir to mix thoroughly.

2. Heat to boiling to dissolve completely.

3. Sterilize in the autoclave at 121ºC. for 15 minutes.

4. Cool to 45-50ºC.

PROCEDURE AND INTERPRETATION OF RESULTS

For information on procedures and interpretation of results, consult listed references or refer to the prepared media Instructions for Use (IFU) for Cat. No. G24.

LIMITATIONS

It is recommended that biochemical, immunological, molecular, or mass spectrometry testing be performed on colonies from pure culture for complete identification.

Some formulations may require a settling period before pH testing of the prepared medium. If the pH is tested immediately after preparation and is out of specification, retest the medium after 24 hours to obtain final pH results.

DNase with Toluidine Blue may be inhibitory to some strains of gram-positive bacteria, particularly staphylococci. When testing for *Branhamella catarrhalis*, a very heavy inoculum must be used.

An inoculum that is too broad may result in complete decolorization of the media, due to the reduction of the dye. If this occurs, the test results must be repeated.

Refer to the document "Limitations of Procedures and Warranty" for more information.

MATERIALS REQUIRED BUT NOT PROVIDED

Standard microbiological supplies and equipment such as autoclaves, incinerators, and incubators, etc., are not provided.

QUALITY CONTROL

Hardy Diagnostics tests each lot of commercially manufactured media using appropriate quality control microorganisms and quality specifications as outlined on the Certificates of Analysis (CofA). The following organisms are routinely used for testing at Hardy Diagnostics:

Test Organisms	Inoculation Method*	Incubation			Results
		Time	Temperature	Atmosphere	
Serratia marcescens ATCC® 8100	E	24-48hr	35°C	Aerobic	Growth; red zone around colonies
Escherichia coli ATCC® 25922	E	24-48hr	35°C	Aerobic	Growth; no color change

* Refer to the document "Inoculation Procedures for Media QC" for more information.

USER QUALITY CONTROL

Users of dehydrated culture media should perform QC testing in accordance with applicable government regulatory agencies, and in compliance with accreditation requirements. Hardy Diagnostics recommends end users check for signs of contamination and deterioration and, if dictated by laboratory quality control procedures or regulation, perform quality control testing to demonstrate growth or a positive reaction and to demonstrate inhibition or a negative reaction, if applicable. Hardy Diagnostics quality control testing is documented on the certificates of analysis (CofA) available from Hardy Diagnostics Certificates of Analysis website. In addition, refer to the following document "Finished Product Quality Control Procedures," for more information on QC or see reference(s) for more specific information.

PHYSICAL APPEARANCE

CRITERION™ DNase Test Agar with Toluidine Blue powder should appear homogeneous, free-flowing, and light bluish-beige in color. The prepared media should appear clear, slightly opalescent, and dark blue in color.

REFERENCES

1. Anderson, N.L., et al. *Cumitech 3B; Quality Systems in the Clinical Microbiology Laboratory,* Coordinating ed., A.S. Weissfeld. American Society for Microbiology, Washington, D.C.

2. Tille, P., et al. *Bailey and Scott's Diagnostic Microbiology,* C.V. Mosby Company, St. Louis, MO.

3. Isenberg, H.D. *Clinical Microbiology Procedures Handbook,* Vol. I, II & III. American Society for Microbiology, Washington, D.C.

4. MacFaddin, J.F. 1985. *Media for Isolation, Cultivation, Identification, Maintenance of Bacteria*, Vol. I. Williams & Wilkins, Baltimore, MD.

5. Jorgensen., et al. *Manual of Clinical Microbiology,* American Society for Microbiology, Washington, D.C.

6. *Quality Assurance for Commercially Prepared Microbiological Culture Media,* M22. Clinical and Laboratory Standards Institute (CLSI - formerly NCCLS), Wayne, PA.

7. Jeffries, Holtman and Guse. 1957. *J. Bacteriol.*; 73:590.

8. Schreier, J.B. 1969. *Am. J. Clin. Pathol.*; 51:711-716.

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The Hardy Diagnostics manufacturing facility and quality management system is certified to ISO 13485.

HDQA 2207B [C]